OIPE JC10 AUG 11 2003 PATENT & TRADEMARK OFFICE

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS P. CERRETTI

Appln. No. 08/538,709 Group Art Unit: 1647

Filed: October 3, 1995 Examiner: Draper, G.

For: DNA ENCODING CYTOKINE DESIGNATED LERK-6

Corrected

DECLARATION UNDER 37 C.F.R. § 1.131

Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

I, DOUGLAS P. CERRETTI do hereby declare and state:

I am the inventor of the invention disclosed and claimed in the above-mentioned application.

I am familiar with U.S. Patent 5,795,734, issued to Flanagan on August 18, 1998 (hereinafter the "Flanagan Patent"), from U.S. Patent Application Serial No. 08/455,001, filed May 31, 1995 (hereinafter the "Flanagan Application"), which I understand is a Continuation-In-Part of Serial No. 08/393,461, filed February 27, 1995 (hereinafter the "Flanagan Parent Application"), which is a Continuation-In-Part of Serial No. 08/308,814, filed September 19, 1994 (hereinafter the "Flanagan Grandparent Application").

The Flanagan Patent discloses DNA encoding Elf-1, a mouse polypeptide which is now known in the art as mouse LERK-6.

In order to demonstrate and establish, *inter alia*, that I conceived and reduced to practice a DNA molecule encoding a polypeptide having mouse LERK-6 sequences in the United States by at least September 15, 1994, i.e., prior to the

September 19, 1994, filing date of the Flanagan Grandparent Application, copies of the following laboratory notebook pages and related materials, identified in detail below, are provided herewith in **Appendices A-G**.

I declare and state that although all of the dates from the laboratory notebook pages and related materials have been removed, all of the dates are prior to at least September 15, 1994.

Appendix A contains pages from Nicole Nelson's Laboratory Notebook No. 4266 (Bates Nos. 0001-0007). At the time, Nicole Nelson was a Research Assistant who worked under my direction and supervision at Immunex Corporation.

Appendix B contains a copy of U.S. Patent 5,516,658 (Bates Nos. 0008-0026).

Appendix C contains oligonucleotide request forms prepared by Carl Kozlosky (Bates Nos. 0027-0030). At the time, Carl Kozlosky was a Research Associate who worked under my direction and supervision at Immunex Corporation.

Appendix D contains pages from Carl Kozlosky's Laboratory Notebook No. 3388 (Bates Nos. 0031-0037).

Appendix E contains various computer printouts of LERK sequences that I personally generated (Bates Nos. 0038-0049).

Appendix F contains the minutes of an internal HEK/ELK meeting at Immunex Corporation that was chaired by Barry Davison in my absence (Bates Nos. 0050-0051). Barry Davison, who prepared the minutes, was the Director of the Transgenics Department at Immunex Corporation at the time.

Appendix G contains a copy of American Type Culture Collection Form BP4/9 for ATCC deposit No. 75829 (Bates No. 0052).

Prior to September 15, 1994, and as described in Example 1 of the present application, a DNA molecule encoding mouse LERK-6 was isolated under my direction and supervision. The specific experiments detailed below were carried out at my behest and command and under my direction and supervision by scientists at Immunex Corporation.

More specifically, prior to September 15, 1994, and as described at page 23, lines 5-7 of the present application, a commercially available 11.5 day murine embryonic cDNA library was obtained by Nicole Nelson from Clonetech Laboratories, Inc., Palo Alto, California (**Appendix A,** Bates No. 0003).

Next, prior to September 15, 1994, and as described on page 23, lines 7-8 of the present application, the library was plated by Nicole Nelson according to the procedures detailed in the manual provided by Clonetech (**Appendix A,** Bates No. 0004). The initial purpose of these efforts was to clone the cDNA for mouse LERK-3 (referred to as A2) and mouse LERK-4 (referred to as C6). However, instead a new mouse LERK molecule was discovered, i.e., mouse LERK-6.

Prior to September 15, 1994, and as described on page 23, lines 8-30 of the present application, probes, referred to as A2 (LERK-3) and C6 (LERK-4), were generated using standard techniques. Generally, polymerase chain reaction (PCR) (Mullis et al, *Meth. Enzymol.*, 155:335-350 (1987)) amplifications were performed by Carl Kozlosky (**Appendix D,** Bates Nos. 0036-0037) using two sets of primers. The first set of primers,

GATATTTACT GCCCGCACTA CAACAGCT	SEQ ID NO:3
AGAGAAGGCG CTGTAGCGCT GGAAC	SEQ ID NO:4

was used to generate amplified double stranded DNA fragments from the DNA of LERK-3 (LERK-3, also known as hek-ligand, is the subject of U.S. Patent 5,516,658 (**Appendix B,** Bates Nos. 0008-0026) which issued from U.S. Patent Application Serial No. 08/240,124, filed May 9, 1994, and which claims benefit of Serial Nos. 08/109,745, 08/114,426 and 08/161,132). The probe from LERK-3 comprised nucleotides 260 through 481 of the SEQ ID NO:1 of U.S. Patent 5,516,658. The second set of primers,

ACGTAGTCTA CTGGAACTCC AGTAACCCCA G SEQ ID NO:5

AGCCTCAAGC ACTGGCCAGA ACTCTCTCTG GAGT SEQ ID NO:6

was used to generate amplified double stranded DNA fragments from the DNA of LERK-4 (LERK-4 also is the subject of U.S. Patent 5,516,658). The probe from LERK-3 comprised nucleotides 110 through 467 of the SEQ ID NO:3 of U.S. Patent 5,516,658.

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:3, i.e., oligo #12334 (also referred to as A2rib5.28) (**Appendix C,** Bates No. 0027), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0027).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:4, i.e., oligo #12333 (also referred to as A2T7.49) (**Appendix C,** Bates No. 0028), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0028).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:5, i.e., oligo #12312

(also referred to as C6RIBO5.31) (**Appendix C,** Bates No. 0029), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0029).

Prior to September 15, 1994, Carl Kozlosky requested that the core facility at Immunex Corporation synthesize the oligonucleotide represented by SEQ ID NO:6, i.e., oligo #12316 (also referred to as C6T7.54) (**Appendix C,** Bates No. 0030), and the core facility actually synthesized such prior to September 15, 1994, as shown in (**Appendix C**, Bates No. 0030).

Oligonucleotides #12333 and 12316 also included nucleotides encoding the T7 polymerase promoter.

Prior to September 15, 1994, Carl Kozlosky identified the location of oligonucleotides #12334 (A2rib5.28) and #12333 (A2T7.49) in the LERK-3 DNA sequence, as shown in **Appendix D,** Bates Nos. 0032-0033); as well as the location of oligonucleotides #12312 (C6RIBO5.31) and 12316 (C6T7.54) in the LERK-4 DNA sequence, as shown **Appendix D,** Bates Nos. 0034-0035).

Thereafter, and prior to September 15, 1994, and as described on page 23, lines 30-35 of the present application, the PCR fragments (A2/LERK-3 and C6/LERK-4) were radiolabelled by Nicole Nelson with ^{32}P (**Appendix A,** Bates No. 0005), and used by Fred Fletcher as probes to screen the murine embryonic cDNA library prepared by Nicole Nelson by conventional procedures, and hybridizing clones were identified. Fred Fletcher was a Staff Scientist at Immunex Corporation at the time who worked on the LERK project under my direction and supervision. The hybridizing conditions consisted of 42°C and 50% Starks washed to 0.1X SSC at 63°C (**Appendix A,** Bates Nos. 0005-0006). In this manner, clone #13 was identified by Fred Fletcher on an X-ray

film, a copy of which was placed in Nicole Nelson's Laboratory Notebook (**Appendix A,** Bates No. 0007).

Then, and prior to September 15, 1994, and as described on page 23, line 35 and page 24, line 4, of the present application, the nucleotide sequence of the cDNA insert of clone #13 (λ13), isolated from the murine embryonic cDNA library, i.e., mouse LERK-6 (mLERK6), was determined at my request by the core facility at Immunex Corporation, and the results were entered into a computer database, a printout of which, which was generated prior to September 15, 1994, is shown in **Appendix E,** Bates Nos. 0038-0039. DNA encoding the first 3 ~~5~~ amino acids shown in **Appendix E** is derived from the sequencing vector, as indicated by the mark between the ~~fifth amino acid (Arg)~~ ninth nucleotide C of CCC and the ~~sixth amino acid (Ala)~~ tenth nucleotide C of CCC. Also, the initiation codon Met is not shown in **Appendix E.** Thus, a substantially complete cDNA sequence of the coding region of the clone λ13 cDNA, and the amino acid sequence encoded thereby were determined, and are presented in SEQ ID NO:1 and SEQ ID NO:2, respectively of the present application. The open-reading frame within this sequence in **Appendix E** (and within SEQ ID NO:1) encodes a protein of 184 amino acids beginning with the ~~second~~ first Ala.

Prior to September 15, 1994, I carried out a comparison of the amino acid sequences of mouse LERK-6 v. human LERK-3 (also referred to as A2) (**Appendix E,** Bates No. 0040); mouse LERK-6 v. human LERK-4 (also referred to as C6) (**Appendix E,** Bates No. 0041); mouse LERK-6 v. human LERK-2 (also referred to as ELKL) (**Appendix E,** Bates No. 0042); mouse LERK-6 v. human LERK-5 (**Appendix E,** Bates No. 0043); mouse LERK-6 v. human LERK-1 (also referred to as B61) (**Appendix E,** Bates No. 0044); mouse LERK-6 v. mouse LERK-4 (also referred to as MC6) (**Appendix E,** Bates

No. 0045); as well as the DNA sequences for mouse LERK-6 v. human LERK-3 (A2) (**Appendix E,** Bates Nos. 0046-0047),); mouse LERK-6 v. mouse LERK-4 (MC6) (**Appendix E,** Bates No. 0048); and mouse LERK-6 v. human LERK-5 (**Appendix E,** Bates No. 0049). These comparisons showed many conserved amino acids and nucleotides amongst the family, and clearly showed that LERK-6 was a member of the LERK family of proteins, and thus would bind to hek/elk.

Prior to September 15, 1994, the results of the above-discussed experiments were presented by Nicole Nelson at an internal HEK/ELK meeting at Immunex Corporation. This meeting was chaired by Barry Davison in my absence, who prepared the meeting minutes, a copy of which is shown in **Appendix F** Bates No. 0050-0051.

Next, as described on page 24, lines 14-17 of the present specification, on July 15, 1994, a cell lysate containing clone λ13 DNA (the LERK-6 cDNA in λgt10) was deposited with the American Type Culture Collection, Rockville, MD, USA, and assigned accession number ATCC 75829. A copy of the deposit receipt is shown in **Appendix G,** Bates No. 0052.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 2/20/01 as corrected 8/7/2003 pg 6

Name: Douglas P. Cerretti
DOUGLAS P. CERRETTI

Douglas P. Cerretti

NOTEBOOK NO. 7266

ISSUED TO Nicole Nelson

ON _____ 19____

DEPARTMENT Nel Sci.

RETURNED _____ 19____

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

IMMUNEX LABORATORY NOTEBOOK

"TABLE OF CONTENTS" FORM

Notebook #: 4266 Date form completed:

Form Completed by: Neil Nelson

MOLECULE(S): c MGF

[redacted]

HEK

Alk

LEAK 4

LEAK 3

PROJECT(S):

Product Analysis Certificate

PRODUCT: Mouse Embryo 5'-STRETCH cDNA Library

CAT. #: ML1027a

LOT #: 1211

STORAGE CONDITIONS:
SHORT-TERM STORAGE (< 6 MONTHS)
4°C

LONG-TERM STORAGE (> 6 MONTHS)
–70°C

SHELF LIFE:
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS:
Dry Ice (–70°C)

PACKAGE CONTENTS:
- 0.2 ml library lysate in 1X Lambda Dilution B and 7% DMSO
- 0.5 ml host strain
- Lambda Library Protocol Handbook (PT10

TITER: $\geq 10^8$ pfu/ml

CLONING VECTOR: λgt10

CLONING SITE: *Eco*R I

PRIMING METHOD: oligo(dT)-primed

HOST STRAIN: C600 *Hfl*

mRNA SOURCE:
whole embryo (not including placenta extraembryonic membranes) from a cross betw ICR outbred females and outbred Swiss We males, 11.5 days post-coitus (noon on the day vaginal plug is 0.5 day post-coitus)

NOTE: No further information on the mRNA sou was made available to CLONTECH.

QUALITY CONTROL DATA

SELECTION CRITERIA: Clear plaques from turbid plaques (nonrecombinar or parental)

ESTIMATED % OF CLEAR PLAQUES: 86%
(when plated on C600 before amplifying in C600*Hfl*

NUMBER OF INDEPENDENT CLONES: 1.7×10^6

AVERAGE INSERT SIZE: 1.5 kb

INSERT SIZE RANGE: 0.8–4.0 kb

AMPLIFICATION: This library was amplified once in C600 *Hfl*

APPROVED BY:

(PA93650-1)

0003

FOR RESEARCH USE ONLY

cDNA for 4-1 Cl

Project No.

Book No.

Page No.

Titer library

0 λ	—		
2 λ	307	1.5×10^{7}	7×10^{7} pfu/λ
5 λ	1572	4.0	
10 λ	13[illegible]	3.4	

2λ library → 1 ml SM → 2λ in 1 ml SM

1:500 1:250000

Plate the cells at 5×10^{4}

$5\times10^{4}/\,$ ... = 13.5 λ $5\times10^{4}/\,4\times10^{4}$ = 1.25 λ

2λ library → 1 ml SM → 10:990 ml SM $5\times10^{4}/\,$ ... = 3.75 λ/plate need 7.5 λ

Take a fresh dilution and plate at 3.75 λ/plate (2). plated by 10 AM

The plates did not grow in 8 hours. I left and returned 11 PM. They had grown to full size & the concentration was ~10 fold low.

0004

To Page No. 8

essed & Understood by me, | Date | Invented by | Date

Recorded by [signature]

Project No.

TITLE 5' Stretch DNA Assay Testing Book No.

From Page No. 83 Probes made for Fred Fletcher to use in Sequencing

```
PROGRAM #=  13   2X Purified probes 12-650 colonies                    01:44
REGION A:   LL-UL=    5-1700   LCR=    0  BKG=     .00  % 2 SIGMA=      .0
REGION B:   LL-UL=   50-1700   LCR=    0  BKG=     .00  % 2 SIGMA=      .0
REGION C:   LL-UL=    0-   0   LCR=    0  BKG=     .00  % 2 SIGMA=      .0
TIME=  1.00  K= 1.000 DIP=SIS

R#   S#   TIME   CPMA/K  %DEV   CPMB/K  %DEV   CPMC/K  %DEV  SIE    SIS FLAGS  MIN
WARNING:  NOT NORMALIZED
 1    1   1.00  481546.  .29  9757.00  2.02     .00   .00  .000  41.366 (60x)   112
 2    2   1.00  666133.  .25 22258.0   1.34     .00   .00  .000  45.390 (10x)   3cc

PROGRAM #=  13                                                         23:32
 REGION A:   LL-UL=    5-1700   LCR=    0  BKG=     .00  % 2 SIGMA=      .0
 REGION B:   LL-UL=   50-1700   LCR=    0  BKG=     .00  % 2 SIGMA=      .0
 REGION C:   LL-UL=    0-   0   LCR=    0  BKG=     .00  % 2 SIGMA=      .0
 TIME=  1.00  K= 1.000 DIP=SIS

 R#   S#   TIME   CPMA/K  %DEV   CPMB/K  %DEV   CPMC/K  %DEV  SIE    SIS FLAGS  MIN
 WARNING:  NOT NORMALIZED
 1    1   1.00  389178.  .32  6839.00  2.42     .00   .00  .000  37.510 (10x)   1 12
 2    2   1.00  293853.  .37  4696.00  2.92     .00   .00  .000  35.396 (10x)   3 cc
```

2 probes tested

1 cc

3 probes tested only

Stretch DNA Assay for AFK Seq. A2

These probes worked well in the moisture controls

included in the hybridization. See Fred Fletcher's files

0005

To Page No. 8

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

1.94

mouse embryo
cDNA library
probed w/ A2, C6
and GST-β.

probed 42°C on stacks

washed to .1XSSC, 63°

1° films
from Fred Fletcher

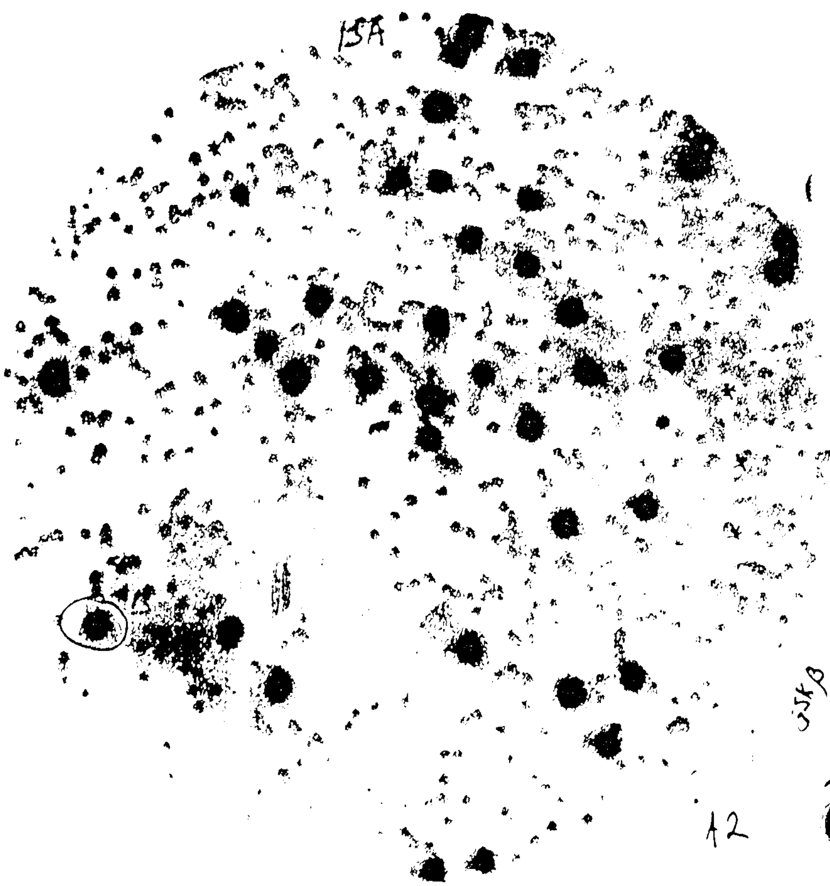
plated this library before xmas 93
but received these films

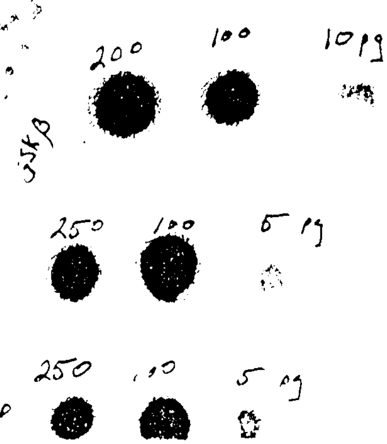
Mu. cDNA 2° clones

Prehyb 42° >2 hrs
Hyb 42° O/N 50% STARKS .7x10^6 cpm/ml A2 & C6 oligo
.7x10^6 cpm/ml G5Kβ riboprobe

Wash RT 6X SSC .1% SDS
63° 1X SSC " 60'
65° 0.1X SSC " 20'

Exp 3 days 4/26/87





Oligo NAME: A2RIB5.28

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

Oligo number: 12334

Date Synthesized:

DNA Sequence (5'-3'): 5'-[illegible] TGC
CCG CAC TAC AAC AGC
T-3'

(28 bases 8A's 4G's
9C's 7T's

PURIFICATION: ~~PHENOL~~ OPC

COMMENTS:
A2 5 PRIME PCR OLIGO FOR
MAKING A T7 RIBOPROBE.

R7043

A2rib5.28

1 GATATTTACT GCCCGCACTA CAACAGCT

Column 2

9:44:32A

Run ID :
Cycle : 40PLUS CYC
End Proc: End CE (DMT = On)
Sequence: 12334

Total bases = 28
A= 8, G= 4, C= 9, T= 7, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 8489.6

5'> GAT ATT TAC TGC CCG CAC TAC AAC AGC T <3'

Purification: OPC
Amount of crude: all
O.D.260: .1686
dilution factor: 1:500
concentration:
yield: 10.09µg/λ
100.9 µg

gel on back

Oligo NAME: A2T7.49 Oligo number: 12333

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-TGC GAA [illegible]
GAC TCA CTA TAG AGA
GAA GGC GCT GTA C G
CTG GAA C-3 '

(49 bases)

16A's 14G's
10C's 9T's

PURIFICATION: ~~PHENOL~~ OPC

CONTENTS:
3 PRIME A2 OLIGO TO PCR
A T7 RIBOPROBE. THIS
OLIGO IS ANTISENSE AND
CONTAINS THE T7
PROMOTER.

A2t7.49

R7044

1 TGCGAATAAT ACGACTCACT ATAGAGAGAA GGCGCTGTAG CGCTGGAAC

Column 1

9:44:31A

Run ID :
Cycle : 40PLUS CYC
End Proc: End CE (DMT = On)
Sequence: 12333

Total bases = 49
A= 16, G= 14, C= 10, T= 9, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 15174.8

5'> TGC GAA TAA TAC GAC TCA CTA TAG AGA GAA GGC GCT GTA

GCG CTG GAA C <3'

Purification: OPC
Amount of crude: all
O.D.260: .276
dilution factor: 1:500
concentration: 4.60 µg/λ
yield: 460 µg

gel on 12334

Oligo NAME: C6RIBO5.31

Oligo number: 12312

Sequence Requested by: KOZLOSKY

Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-ACG TAG TCT ACT GGA ACT CCA GTA ACC CCA G-3'

(31 bases)

9A's 6G's
10C's 6T's

PURIFICATION: ~~[illegible]~~ OPC

COMMENTS:
5 PRIME PCR FOR C6 RIBO

R7023

Column I

3:45:43P

ur ID :
ycle : 40PLUS C:C
nd Proc: End CE (DMT = On)
equence: 12312

Applied Biosystems G 209118

otal bases = 31
A= 9, G= 6, C= 10, T= 6, 5= 0, 6= 0, 7= 0, 8= 0
(mixed bases= 0)

W: 9444.2

5' ACG TAG TCT ACT GGA ACT CCA GTA ACC CCA G 3'

Purification: OPC

Amount of crude: all

O.D.260: 0.382

dilution factor: 1:500

concentration: 6.36 ug/x

yield: 636 ug

gel on 12,334

Oligo NAME: C6T7.54

Oligo number: 12316

Sequence Requested by: KOZLOSKY
Project name: ELK

Date Requested:

Date Synthesized:

DNA Sequence (5'-3'): 5'-TGC GAA TAA TAC
GAC TCA CTA TAG CCT
CAA GCA CTG GCC AGA
ACT CTC TGG AGT -3'

(54 bases) 16A's 11G's
15C's 12T's

PURIFICATION: ~~PHENOL~~ OPC

COMMENTS:
C6 3 PRIME FOR C6 RIBO
USE T7 POL.

Applied Biosystems T 453741

R7024

COLUMN 2 SET-UP
VERSION 2.02

USER_NAME:
CYCLES USED: 0.2UMB - 1
ENDING METHOD: Trityl ON, Auto
ENDING PROCEDURE: deprce
SEQUENCE NAME: 12316
SEQUENCE LENGTH: 54
DATE:
TIME: 17:37
COMMENT:

5'- TGC GAA TAA TAC GAC TCA CTA TAG CCT CAA GCA CTG

GCC AGA ACT CTC TGG AGT -3'

yield:

OPC
all
0.303
1:50
5.04 ug/x
504 ug

gel on 12334

IMMUNEX LABORATORY NOTEBOOK

"TABLE OF CONTENTS" FORM

Notebook #: 3388 Date form completed:

Form Completed by: Carl Kozlosky

MOLECULE(S): B61, ELK, ELK-L, HPK, Lorks 1, 2, 3, 4, 5, 7

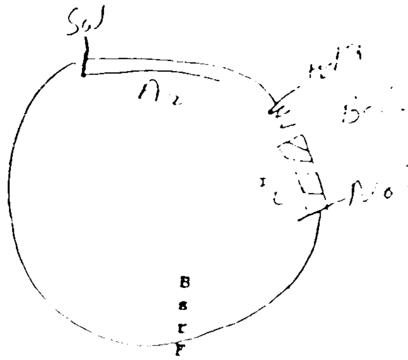
PROJECT(S): RISOL

(Linear) (Six Base) MAP of: A2.Seq check: 6473 from: 1 to: 1037

HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL]

With 114 enzymes: *

T7 is 5' End
Bgl2 into BamHI



09:13 ..

Xho2 Esp31 BspM1 Bpm1 BspB1a2n826 BsrF1 NspB2

1 GGATCTTGGAACGAGACGACCTGCTGGAGAAGCCGGGAGCGCGGGGCTCAGTCGGGGGGCGGCGGCGGCGGCGGCTCCGGGGATGGCGGCGGCTCCGCTG
CCTAGAACCTTGCTCTGCTGGACGACCTCTTCGGCCCTCGCGCCCCGAGTCAGCCCCCCGCCGCCGCCGCCGCCGAGGCCCCTACCGCCGCCGAGGCGAC 100

AspLeuGlyThrArgArgProAlaGlyGluAlaGlySerAlaGlyLeuSerArgGlyAlaAlaAlaAlaAlaAlaProGlyMetAlaAlaAlaProLeu -

Bsp1286 NspB2 NspB2 Sty1 Dra2 BsaABrpaFan11 Bsp1286 Hae2 NspSphH11

101 CTGCTGCTGCTGCTGCTCGTGCCCGTGCCGCTGCTGCCGCTGCTGGCCCAAGGGCCCGGAGGGGCGCTGGGAAACCGGCATGCGGTGTACTGGAACAGCT
GACGACGACGACGACGAGCACGGGCACGGCGACGACGGCGACGACCGGGTTCCCGGGCCTCCCCGCGACCCTTTGGCCGTACGCCACATGACCTTGTCGA 200

LeuLeuLeuLeuLeuLeuValProValProLeuLeuProLeuLeuAlaGlnGlyProGlyGlyAlaLeuGlyAsnArgHisAlaValTyrTrpAsnSerSer -

AlwN1 BspM1 BspM1 Ava1 Dra2 AApvaa11

A2R185.28

201 CCAACCAGCACCTGCGGCGAGAGGGCTACACCGTGCAGGTGAACGTGAACGACTATCTGGATATTTACTGCCCGCACTACAACAGCTCGGGGGTGGGCCC
GGTTGGTCGTGGACGCCGCTCTCCCGATGTGGCACGTCCACTTGCACTTGCTGATAGACCTATAAATGACGGGCGTGATGTTGTCGAGCCCCCACCCGGG 300

AsnGlnHisLeuArgArgGluGlyTyrThrValGlnValAsnValAsnAspTyrLeuAspIleTyrCysProHisTyrAsnSerSerGlyValGlyPro -

BspB1a2n826 BsrF PSX smm maa 11 Dra2 BsaAB rpa Fan11 Bsp1286 BsaA1 Bgl1 BspM1

301 CGGGGCGGGACCGGGGCCCGGAGGCGGGGCAGAGCAGTACGTGCTGTACATGGTGAGCCGCAACGGCTACCGCACGTGCAACGCCAGCCAGGGCTTCAAG
GCCCCGCCCTGGCCCCGGGCCTCCGCCCCGTCTCGTCATGCACGACATGTACCACTCGGCGTTGCCGATGGCGTGGACGTTGCGGTCGGTCCCGAAGTTC 400

GlyAlaGlyProGlyProGlyGlyGlyAlaGluGlnTyrValLeuTyrMetValSerArgAsnGlyTyrArgThrCysAsnAlaSerGlnGlyPheLys -

Eco473 Hae2 Eco473 HSaef ec21 Hae2 NgoM1

A2T7.49

401 CGCTGGGAGTGCAACCGCCCGCACGCGCCGCACAGCCCCATCAAGTTCTCGGAGAAGTTCCAGCGCTACAGCGCCTTCTCTCTGGGCTACGAGTTCCACG
GCGACCCTCACGTTGGCGGGCGTGCGCGGCGTGTCGGGGTAGTTCAAGAGCCTCTTCAAGGTCGCGATGTCGCGGAAGAGAGACCCGATGCTCAAGGTGC 500

ArgTrpGluCysAsnArgProHisAlaProHisSerProIleLysPheSerGluLysPheGlnArgTyrSerAlaPheSerLeuGlyTyrGluPheHisAla -

Eae1 Sca1 BspM1 Xmn1 Bsr

CCGGCCACGAGTACTACTACATCTCCA...

0032

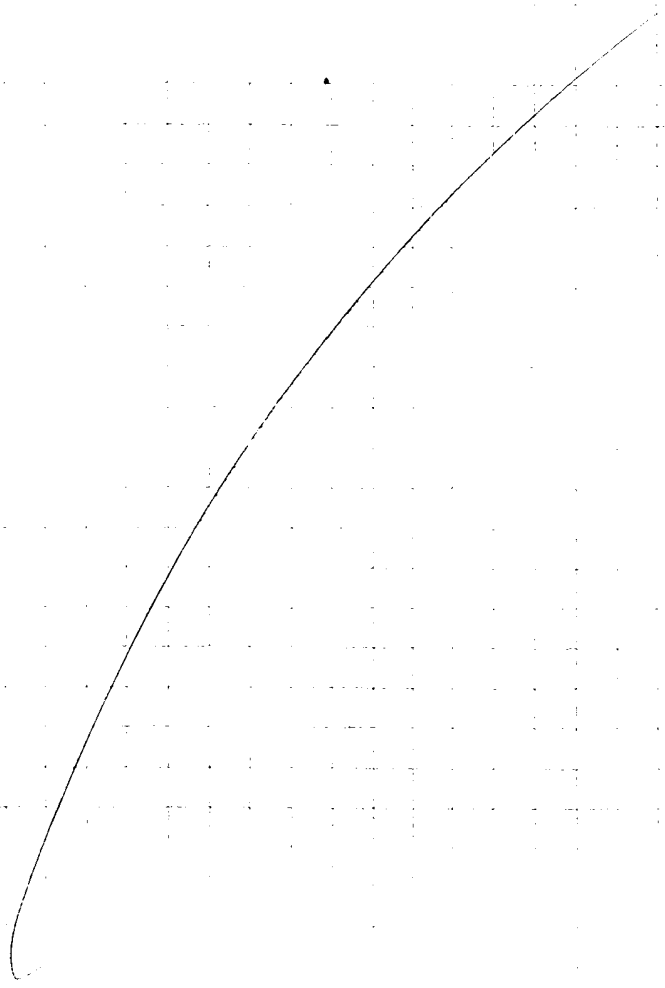
TITLE A2 Seq.

Project No.

Book No.

62

From Page No.



To Page No.

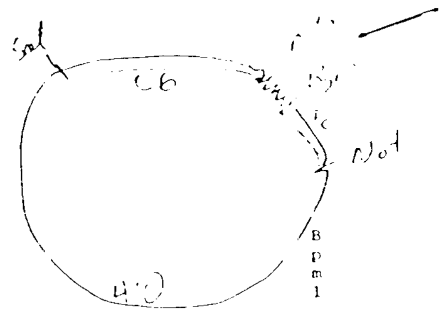
Witnessed & Understood by me,	Date	Invented by	Date
apc		Recorded by	

(Linear) (Six Base) MAP of: C eq check: 9352 from: 1 to: 636

HSKL 132-11, C6-no vector
2491,T7,DPC3266,DPC3267,DPC3274,DPC3275
SR1810 KOZLOSKY
file: [BERTLESJ.HEKL]C6.SEQ

With 114 enzymes: *

12:12 ..




```
          A   B                                                                      B         B
          v   c                                                                      p         s
          a   g                                                                      m         p
          1   1                                                                      1         B1
                                                                                               a2
                                                                                               n8
                                                                                               26
                                                                                               /
   GCCAGACCAAACCGGACCTCGGGGGCGATGCGGCTGCTGCCCCTGCTGCGGACTGTCCTCTGGGCCGCGTTCCTCGGCTCCCCTCTGCGCGGGGGCTCCA
1  ---------------------------------------------------------------------------------------------------- 100
   CGGTCTGGTTTGGCCTGGAGCCCCCGCTACGCCGACGACGGGGACGACGCCTGACAGGAGACCCGGCGCAAGGAGCCGAGGGGAGACGCGCCCCCGAGGT

a: AlaArgProAsnArgThrSerGlyAlaMetArgLeuLeuProLeuLeuArgThrValLeuTrpAlaAlaPheLeuGlySerProLeuArgGlyGlySerSer

     B
     Bs   A                          E         B  D                          T
     pa   c                          s         s  s                          t
     mA   c                          p         a  a                          h
     11   1                          3         H  1                          3
     /                               1         1                             1
   GCCTCCGCCACGTAGTCTACTGGAACTCCAGTAACCCCAGGTTGCTTCGAGGAGACGCCGTGGTGGAGCTGGGCCTCAACGATTACCTAGACATTGTCTG
101 --------------------------------------------------------------------------------------------------- 200
   CGGAGGCGGTGCATCAGATGACCTTGAGGTCATTGGGGTCCAACGAAGCTCCTCTGCGGCACCACCTCGACCCGGAGTTGCTAATGGATCTGTAACAGAC

a: LeuArgHisValValTyrTrpAsnSerSerAsnProArgLeuLeuArgGlyAspAlaValValGluLeuGlyLeuAsnAspTyrLeuAspIleValCys

                    B         B
                    sB        s
                    ps    E   p
             DD  PABluP  DDsPAABB1P            E B                        DD PAAB
             rr  spa23s  rrpspvac2s            a a                        rr spva
             aa  san86s  aa3saang8s            e l                        aa saan
             22  112611  2211112161            1 1                        22 1112
                 /// /    /  // //                                           /
   CCCCCACTACGAAGGCCCAGGGCCCCCTGAGGGCCCCGAGACGTTTGCTTTGTACATGGTGGACTGGCCAGGCTATGAGTCCTGCCAGGCAGAGGGCCCC
201 --------------------------------------------------------------------------------------------------- 300
   GGGGGTGATGCTTCCGGGTCCCGGGGGACTCCCGGGGCTCTGCAAACGAAACATGTACCACCTGACCGGTCCGATACTCAGGACGGTCCGTCTCCCGGGG

a: ProHisTyrGluGlyProGlyProProGluGlyProGluThrPheAlaLeuTyrMetValAspTrpProGlyTyrGluSerCysGlnAlaGluGlyPro

   B                    B
   s                    s                                      E
   pB A    E            pH                                     c
   1sBSX   c            1g                                     o H
   2rsmm   o H          2i       E B                           4 a
   8Fsaa   4 a          8A       a a                           7 e
   61111   7 e          61       e l                           3 2
   ////    3 2          /        1 1
   CGGGCCTACAAGCGCTGGGTGTGCTCCCTGCCCTTTGGCCATGTTCAATTCTCAGAGAAGATTCAGCGCTTCACACCTTTCTCCCTCGGCTTTGAGTTCT
301 --------------------------------------------------------------------------------------------------- 400
   GCCCGGATGTTCGCGACCCACACGAGGGACGGGAAACCGGTACAAGTTAAGAGTCTCTTCTAAGTCGCGAAGTGTGGAAAGAGGGAGCCGAAACTCAAGA

a: ArgAlaTyrLysArgTrpValCysSerLeuProPheGlyHisValGlnPheSerGluLysIleGlnArgPheThrProPheSerLeuGlyPheGluPheLeu

                                  B
                                  s
                                  p
                      B  B   B    1             T
                      p  p   a    2             t   EBB
                      m  m   n    8             h   apa
                      1  1   1    6             3   eml
                                                2   111
   TACCTGGAGAGACTTACTACTACATCTCGGTGCCCACTCCAGAGAGTTCTGGCCAGTGCTTGAGGCTCCAGGTGTCTGTCTGCTGCAAGGAGAGGAAGTC
401 --------------------------------------------------------------------------------------------------- 500
   ATGGACCTCTCTGAATGATGATGTAGAGCCACGGGTGAGGTCTCTCAAGACCGGTCACGAACTCCGAGGTCCACAGACAGACGACGTTCCTCTCCTTCAG

a  ProGlyGluThrTyrTyrTyrIleSerValProThrProGluSerSerGlyGlnCysLeuArgLeuGlnValSerValCysCysLysGluArgLysSer

                  B
                  s
        B         p
        s         B1                   B
        t         a2                   p
        y         n8                   m
        1         26                   1
                  /
   TGAGTCAGCCCATCCTGTTGGAGCCCTGGAGAGAGTGGCACATCAGGGTGGCGAGGGGGGGACACTCCCAGCCCCCTCTGTCTCTTGCTATTACTGCTG
501 --------------------------------------------------------------------------------------------------- 600
   ACTCAGTCGGGTAGGACAACCCTCGGGACCTCTCTCACCGTGTAGTCCCACCGCTCCCCCCCTGTGAGGGTCGGGGGAGACAGAGAACGATAATGACGAC
```

C6RIBO.31

C6T7.54

GATATCAGTCAGCATAATAAGCGT

TCTAG

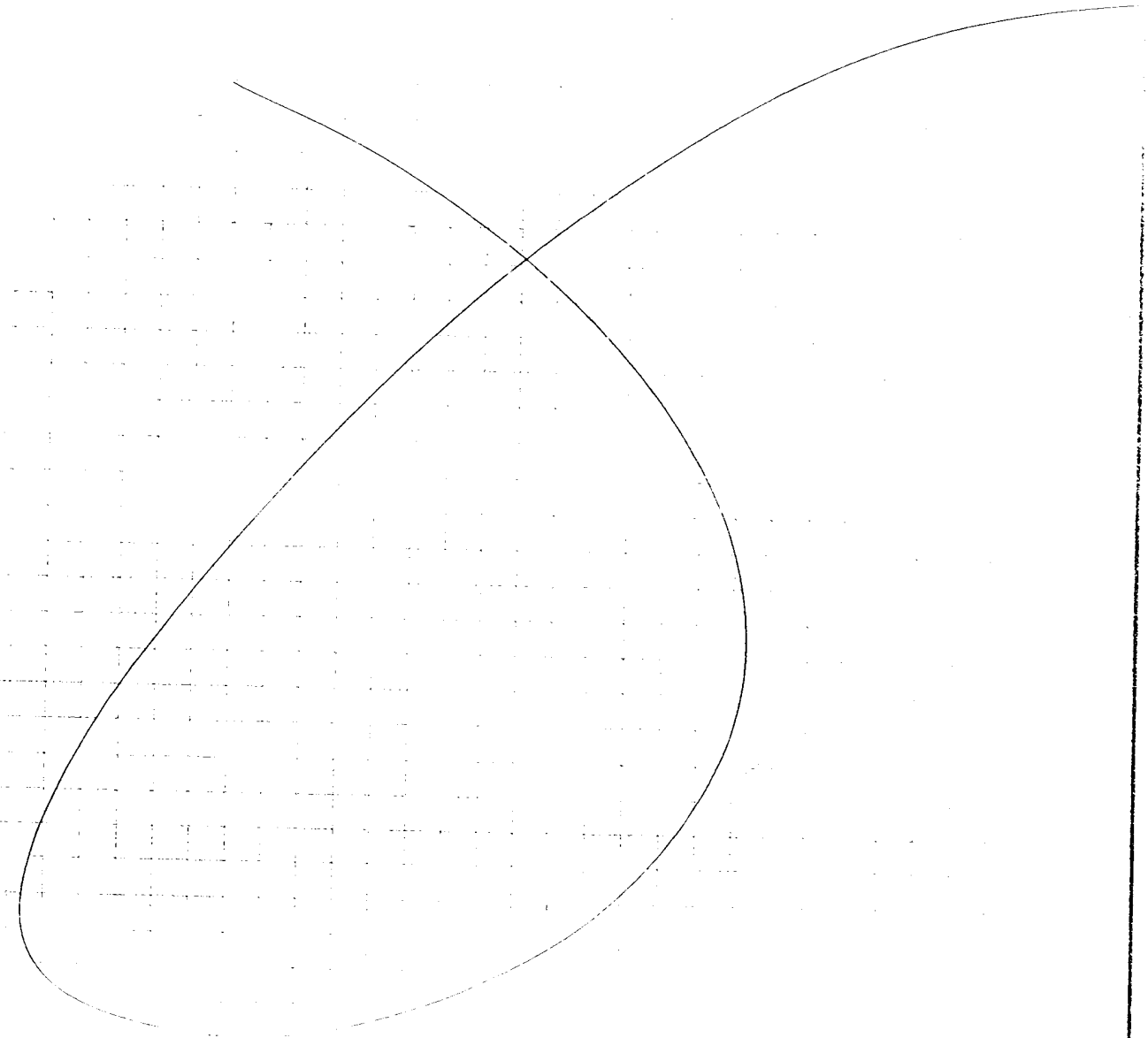
179 aa ≈ 20 kd

TITLE Cb Sag.

Project No.

Book No.

From Page No.



To Page No.

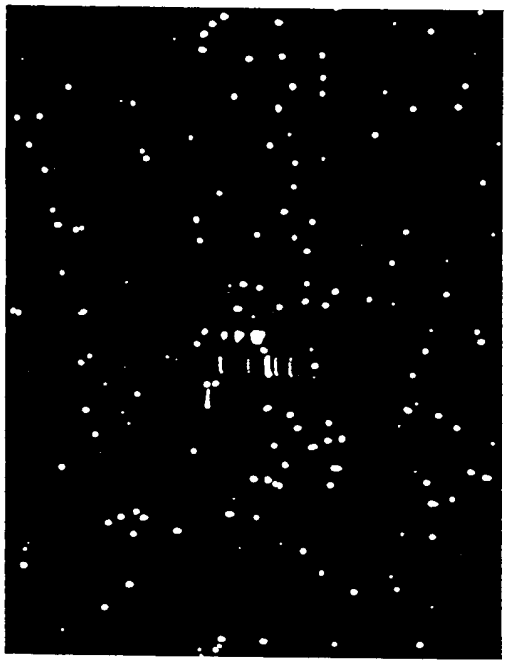
Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	

TITLE C6 T7 Ribo PCR

Project No.

Book No.

From Page No.



C6 Binding Region
T7 RNA Pol

To Page No.

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by	

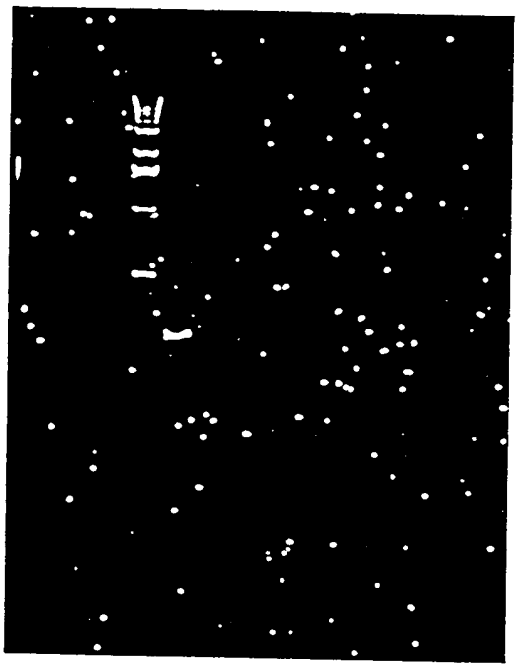
TITLE Clb 1th

Project No.

Book No.

From Page No.

< 189 ~ 1.6Kb in HSB-2



New A2 T7
Probe
Template

To Page No.

Witnessed & Understood by me.	Date	Invented by	Date
		Recorded by	

(Linear) (Six Base) MAP of: ... Check: ... from: 1 to: ...

Working File
Do Not Copy!

With 114 enzymes: *

13:38

```
                    B
                    s
                    pB                                                           E
  E   B           AAB1sSSX                   A                                   c
  Ac  s           vpa2rmrm                   c                                   o H
  po  r           aan8Fafa                   c                                   4 a
  oR  F           11261111                   1                                   7 e
  11  1                                                                          3 2
   /               //////
  GAATTCCGGGCCCGGGCCAACGCTGACCGATACGCAGTCTACTGGAACCGTAGCAACCCCAGGTTTCAGGTGAGCGCTGTGGGTGATGGCGGCGGCTATA
1 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 100
  CTTAAGGCCCGGGCCCGGTTGCGACTGGCTATGCGTCAGATGACCTTGGCATCGTTGGGGTCCAAAGTCCACTCGCGACACCCACTACCGCCGCCGATAT

a:  GluPheArgAlaArgAlaAsnAlaAspArgTyrAlaValTyrTrpAsnArgSerAsnProArgPheGlnValSerAlaValGlyAspGlyGlyGlyTyrThr -

                                                                                 B
                                                                                 p
                                                                                 u
                                          E                                      1
  D                                       c                    B    N            1
  s                                       o                  BsKNH  s            0
  a                                       R                  aaaaa  p            2
  1                                       5                  nHsre  B            1
                                                             11112  2
                                                               //
    CCGTGGAGGTGAGCATCAACGACTACCTGGATATCTACTGCCCACACTACGGGGCGCCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTACAT
101 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 200
    GGCACCTCCACTCGTAGTTGCTGATGGACCTATAGATGACGGGTGTGATGCCCCGCGGCGACGGGGGCCGACTCGCGTACCTCGCCATGTAGGACATGTA

a:  ValGluValSerIleAsnAspTyrLeuAspIleTyrCysProHisTyrGlyAlaProLeuProProAlaGluArgMetGluArgTyrIleLeuTyrMet -

                                                             E
                                                             c
                                                             o H          B          B      P
                                                             4 a          s        AsHSX Dp P
                                                             7 e          m        vramm ru s
                                                             3 2          1        aFeaa aM s
                                                                                   11211 21 1
                                                                                    ////  /
    GGTGAATGGTGAGGGCCACGCCTCCTGTGACCACCGGCAGCGAGGCTTCAAGCGCTGGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCAAGTTC
201 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 300
    CCACTTACCACTCCCGGTGCGGAGGACACTGGTGGCCGTCGCTCCGAAGTTCGCGACCCTTACGTTGGCCGGGCGTCGCGGGCCCCCTGGGGAGTTCAAG

a:  ValAsnGlyGluGlyHisAlaSerCysAspHisArgGlnArgGlyPheLysArgTrpGluCysAsnArgProAlaAlaProGlyGlyProLeuLysPhe -

                         E                                                                          B
                         a                                  E B                                     s
                         r                                  a a                                     t
                         1                                  e l                                     X
                                                            1 1                                     1
    TCAGAGAAGTTCCAACTCTTCACCCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGGCCACGAATACTACTACATCTCTGCCACACCTCCCAACCTCGTGG
301 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 400
    AGTCTCTTCAAGGTTGAGAAGTGGGGGAAAAGGGACCCGAAACTCAAGGCCGGACCGGTGCTTATGATGATGTAGAGACGGTGTGGAGGGTTGGAGCACC

a:  SerGluLysPheGlnLeuPheThrProPheSerLeuGlyPheGluPheArgProGlyHisGluTyrTyrTyrIleSerAlaThrProProAsnLeuValAsp -

                                                                          B
                                                                          s
                                                            E             p                      A      N
                                              B      B      c            B1                      1S     Ps
                                              s      p      o            a2                      wf     sp
                                              a      m      N            n8                      Nc     tB
                                              1      1      1            26                      11     12
                                                                          /
    ACCGACCCTGCCTGCGACTCAAGGTTTATGTGCGTCCAACCAATGAGACCCTGTATGAGGCTCCAGAGCCCATCTTCACCAGTAACAGCTCCTGCAGCGG
401 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 500
    TGGCTGGGACGGACGCTGAGTTCCAAATACACGCAGGTTGGTTACTCTGGGACATACTCCGAGGTCTCGGGTAGAAGTGGTCATTGTCGAGGACGTCGCC

a:  ArgProCysLeuArgLeuLysValTyrValArgProThrAsnGluThrLeuTyrGluAlaProGluProIlePheThrSerAsnSerSerCysSerGly

                                                               B
                                                               .
                                                               P
      B                  E                                     B1
      g                  a                                     a2
      l                  r                                     n8
      1                  1                                     26
                                                                /
    CCTGGGTGGCTGCCACCTCTTCCTCACCACCGTCCCTGTGCTGTGGTCCCTTCTGGGCTCCTAGTGTCAGGCCGGAGAACACCAGCCCCACCTGGACCCC
501 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 600
    GGACCCACCGACGGTGGAGAAGGAGTGGTGGCAGGGACACGACACCAGGGAAGACCCGAGGATCACAGTCCGGCCTCTTGTGGTCGGGGTGGACCTGGGG

a:  LeuGlyGlyCysHisLeuPheLeuThrThrValProValLeuTrpSerLeuLeuGlySerEndCysGlnAlaGlyGluHisGlnProHisLeuAspPro -
```

```
                                  B
                              D  Es
                              s  ap
                              a  eM
                              1  11
        GTGACCTTTGCCCTCTGACCTGCCACGGCCACCTCCGAGACAAAATCCTTGCTGCTTCTCTTTCATGGTGCTGTCCCGCCGGAGGAGGCCATCCATCCGT
    601 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 700
        CACTGGAAACGGGAGACTGGACGGTGCCGGTGGAGGCTCTGTTTTAGGAACGACGAAGAGAAAGTACCACGACAGGGCGGCCTCCTCCGGTAGGTAGGCA

a:      ValThrPheAlaLeuEndProAlaThrAlaThrSerGluThrLysSerLeuLeuLeuLeuPheHisGlyAlaValProProGluGluAlaIleHisProSer -

                                     B
                           P         s
                          Dp P       u             B    S
                          ru s       3             b    t                              BS
                          aM s       6             s    y                              gf
                          21 1       1             1    1                              li
                           /                                                           11
                                                                                        /
        CCCTGGGATGCAACATGGGGTCCCAATGCCTGAGGAGAAGACCCCCCCCCAAGGCTGACTCGCTTTCACCAGGGCCACCAGGGCCATCCAGTGTTGYATA
    701 ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+ 800
        GGGACCCTACGTTGTACCCCAGGGTTACGGACTCCTCTTCTGGGGGGGGGTTCCGACTGAGCGAAAGTGGTCCCGGTGGTCCCGGTAGGTCACAACRTAT

a:        LeuGlyCysAsnMetGlySerGlnCysLeuArgArgArgProProProLysAlaAspSerLeuSerProGlyProProGlyProSerSerVal???End -

        ATTCTTT
    801 ------- 807
        TAAGAAA

a:       PhePhe   -
```

Enzymes that do cut:

Acc1	AlwN1	Apo1	Apa1	Ava1	Bal1	Ban1	Ban2	Bbs1	Bgl1	Bpu1102l	Bpm1	Bsa1
BsaH1	Bsm1	Bsp1286	BspM1	BsrF1	BstX1	Bsu361	Dra2	Dsa1	Eae1	Ear1	Eco473	EcoN1
EcoR1	EcoR5	Hae2	Kas1	Nar1	NspB2	PpuM1	Pss1	Pst1	Sfc1	Sfi1	Sma1	Srf1
Sty1	Xma1											

Enzymes that do not cut:

Aat2	Afl2	Afl3	Age1	ApaL1	Asc1	Ase1	Asp718	Asu2	Avr2	BamH1	Bcg1	Bcl1
Bgl2	BsaA1	BsaB1	BsiE1	BsiW1	BspE1	BspH1	BssH2	Bst1107	BstE2	Cla1	Dra1	Dra3
Drd1	Eam1105	Eco571	Esp31	Fsp1	HgiA1	Hinc2	Hind3	Hpa1	Kpn1	Mlu1	Mun1	Nco1
Nde1	NgoM1	Nhe1	Not1	Nru1	Nsi1	NspH1	Pac1	PflM1	Pme1	Pml1	Pvu1	Pvu2
Rsr2	Sal1	Sca1	SgrA1	SnaB1	Spe1	Sph1	Sse8387	Ssp1	Sst1	Sst2	Stu1	Swa1
Tth31	Tth32	Xba1	Xcm1	Xho1	Xho2	Xma3	Xmn1					

```
GAP of: Mlerk6.Pep  check:  6430  from: 1  to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

 to: A2.Pep  check: 4723  from: 1  to: 238

TRANSLATE of: a2.seq check: 6473 from: 83 to: 796
generated symbols 1 to: 238.
HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL] . . .

 Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
 CompCheck: 1254

        Gap Weight:  3.000      Average Match:  0.540
     Length Weight:  0.100   Average Mismatch: -0.396

           Quality:  137.9             Length:    246
             Ratio:  0.741               Gaps:      6
 Percent Similarity: 67.416   Percent Identity: 48.876

 Mlerk6.Pep x A2.Pep                    16:30  ..

       1 .......................RARANADRYAVYWNRSNPRFQVSAVG  26
                                .: | ::|.||||||.||.::.
       1 MAAAPLLLLLLLVPVPLLPLLAQGPGGALGNRHAVYWNSSNQHLRR....  46

      27 DGGGYTVEVSINDYLDIYCPHY.......GAPLPPAERMERYILYMVNGE  69
           :|||||.|.:||||||||||||       ||. .|::  |.|:||||. :
      47 ..EGYTVQVNVNDYLDIYCPHYNSSGVGPGAGPGPGGGAEQYVLYMVSRN  94

      70 GHASCDHRQRGFKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHE 119
         |. .|: .| |||||||||| || :|:|||||||  :.:||||:||::|||
      95 GYRTCNASQ.GFKRWECNRPHAPHSPIKFSEKFQRYSAFSLGYEFHAGHE 143

     120 YYYISATPPNLVDRPCLRLKVYV......RPTNETLYEAPEPIFTSNSSC 163
         ||||| ||.: :.:.|||:||:|      :..:..: . |: .:..| ..
     144 YYYIS.TPTHNLHWKCLRMKVFVCCASTSHSGEKPVPTLPQFTMGPNVKI 192

     164 SGLGGCHLFLTTVPVL.WSLLGS*..................... 186
         ..|:: .   . || | .|: |.
     193 NVLEDFEGENPQVPKLEKSISGTSPKREHLPLAVGIAFFLMTFLAS 238
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: C6.Pep check: 8194 from: 1 to: 201

TRANSLATE of: c6.seq check: 6086 from: 53 to: 655
generated symbols 1 to: 201.
HEKL 132-11, C6-no vector
2491,T7,DPC3266,DPC3267.DPC3274,DPC3275
SR1810 KOZLOSKY
file: [BERTLESJ.HEKL]C6.SEQ . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	118.5	Length:	216
Ratio:	0.637	Gaps:	7
Percent Similarity:	61.988	Percent Identity:	46.199

Mlerk6.Pep x C6.Pep 16:31 ..

```
      1 ..................RARANAD.RYAVYWNRSNPRFQVSAVGDGGGY  31
                           . |:... |..|||| .|||| :        |:
      1 MRLLPLLRTVLWAAPLGSPLRGGSSLRHVVYWNSSNPRLL......RGDA  44

     32 TVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCD.HRQRG  80
        .||:::|||||| |||| ::| ||.:. | : |||| : .|..||: . .|:
     45 VVELGLNDYLDIVCPHYEGPGPPEGP.ETFALYMVDWPGYESCQAEGPRA  93

     81 FKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNL 130
        :||| |   . | |.:.|||| :| |||||||||| ||..||||...|:
     94 YKRWVC...SLPFGHVQFSEKIQRFTPFSLGFEFLPGETYYYISVPTPES 140

    131 VDRPCLRLKVYVRPTNETLYEAPEPIFTSNSSCSGLGGCH.......... 170
         :. |||| .| | ..: .  .|...  .::|:.||: |..
    141 SGQ.CLRLQVSVCCKERKSESAHPVGSPGESGTSGWRGGDTPSPLCLLLL 189

    171 LFLTTVPVLWSLLGS* 186
        |:| .:.:|: |
    190 LLLLILRLLRIL.... 201
```

GAP of: Mlerk6.Pep che : 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: Elkl.Pep check: 1665 from: 1 to: 240

TRANSLATE of: tele7.seq check: 2210 from: 308 to: 1345
generated symbols 1 to: 346.
[hollingsworth.tele7] ELKL-E7.SEQ + ELKL-E7-3PRIME.SEQ;
req#1262
mGel 97 #2491+ #2492-/ mGel101 DPC2236+ DPC2239+/ mGel104 DPC2258+
DPC2257-/mGel105 DPC2261- /mGel107 DPC2271+ 2272- 2273- 2274+ . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	82.7	Length:	248
Ratio:	0.445	Gaps:	6
Percent Similarity:	46.067	Percent Identity:	28.652

Mlerk6.Pep x Elkl.Pep 16:46 ..

```
                .         .         .         .         .
  1 RARANADR.........YAVYWNRSNPRFQVSAVG.........DGGGY 31
    .||:.. :          :|:::  .    .:.:|:          .| |.
  1 MARPGQRWLGKWLVAMVVWALCRLATPLAKNLEPVSWSSLNPKFLSGKGL 50
                .         .         .         .         .
 32 TVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQRGF 81
    .: ..|.| |||.||: :|. |     | | ||:|..|. |.|.    .
 51 VIYPKIGDKLDIICPRAEAGRP....YEYYKLYLVRPEQAAACSTVLDPN 96
                .         .         .         .         .
 82 KRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNLV 131
       .|||   |:..::|. ||| |.|  :|:||:. |:||..|.. ..|
 97 VLVTCNR...PEQEIRFTIKFQEFSPNYMGLEFKKHHDYYITSTSNGSLE 143
                .         .         .         .         .
132 D........RPCLRLKVYVRPTNETLYEAPEPIFTSNSSCSGLGGCHLFL 173
    :          ..  :|:.::...:.   .||.: ||..| .: ....:
144 GLENREGGVCRTRTMKIIMKVGQDPNAVTPEQLTTSRPSKEADNTVKM.A 192
                .         .         .         .
174 TTVPVLWSLLGS*.............................. 186
    | .|. :: ||.
193 TQAPGSRGSLGDSDGKHETVNQEEKSGPGASGGSSGDPDGFFNSKVAL 240
```

GAP of: Mlerk6.Pep check: 6430 from: 1 to: 186

TRANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: Lerk5.Pep check: 8553 from: 1 to: 240

TRANSLATE of: lerk5.leg check: 889 from: 1 to: 1002
generated symbols 1 to: 334.
Coding region of human LERK-5.

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	83.2	Length:	250
Ratio:	0.447	Gaps:	5
Percent Similarity:	47.727	Percent Identity:	27.841

Mlerk6.Pep x Lerk5.Pep 16:59 ..

```
      1 ..................RARANADRY....AVYWNRSNPRFQVSAVGDG  28
                           .|.. .:     ::|||.||.:|     .|
      1 MAVRRDSVWKYCWGVLMVLCRTAISKSIVLEPIYWNSSNSKFL.....PG  45

     29 GGYTVEVSINDYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQ  78
        .|..: . |.| |||.||. :.  ...:. | | :|||: :. ..|. :.
     46 QGLVLYPQIGDKLDIICPKVDS..KTVGQYEYYKVYMVDKDQADRCTIKK  93

     79 RGFKRWECNRPAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPP 128
          . . ::|   | |:..:||. ||| |.|  :|:||....:||.||.. .
     94 ENTPLLNC...AKPDQDIKFTIKFQEFSPNLWGLEFQKNKDYYIISTSNG 140

    129 NLVD.....................................RPCLRLKVY 141
        .| :                                    || |   .
    141 SLEGLDNQEGGVCQTRAMKILMKVGQDASSAGSTRNKDPTRRPELEAGTN 190

    142 VRPTNETLYEAPEPIFTSNSSCSGLGGCHLFLTTVPVLWSLLGS*..... 186
        .|..... :  |:|  ..:::.:.| :| ::: ..|::: :: ::
    191 GRSSTTSPFVKPNPGSSTDGNSAGHSGNNILGSEVALFAGIASGCIIFIV 240
```

Mlerk6.Pep check: 6430 from: 1 to: 18

RANSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
DO NOT COPY!

to: B61.Pep check: 4381 from: 1 to: 205

TRANSLATE of: b61.seq check: 6304 from: 74 to: 688
generated symbols 1 to: 205.
LOCUS HUMB61 1480 bp ss-mRNA PRI
DEFINITION Human B61 mRNA, complete cds.
ACCESSION M57730 M37476
KEYWORDS intermediate-early response gene. . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	128.5	Length:	212
Ratio:	0.691	Gaps:	4
Percent Similarity:	59.218	Percent Identity:	45.251

Mlerk6.Pep x B61.Pep 16:29 ..

```
      .         .         .         .         .
  1 ...........RARANADRYAVYWNRSNPRFQVSAVGDGGGYTVEVSIN 38
                . |.|||..|:||.|||:|.      .::||:.| :|
  1 MEFLWAPLLGLCCSLAAADRHTVFWNSSNPKFR......NEDYTIHVQLN 44
      .         .         .         .         .
 39 DYLDIYCPHYGAPLPPAERMERYILYMVNGEGHASCDHRQRGFKRWECNR 88
    ||:||.||||:.. .:.. ||.||||:|: |:.. |:.. ::  ||:|||
 45 DYVDIICPHYEDHSVADAAMEQYILYLVEHEEYQLCQPQSKDQVRWQCNR 94
      .         .         .         .         .
 89 PAAPGGPLKFSEKFQLFTPFSLGFEFRPGHEYYYISATPPNLVDRPCLRL 138
    |.|. || |:||||| ||||.|| ||:.||.||||| . ..  || ||||
 95 PSAKHGPEKLSEKFQRFTPFTLGKEFKEGHSYYYISKPIHQHEDR.CLRL 143
      .         .         .         .         .
139 KVYVRP...........TNETLYEAPEPIFTSNSSCSGLGGCHLF.LTTV 176
    || |..           ..|. ..|.:| .    |.:  ::.:|| |. .
144 KVTVSGKITHSPQAHVNPQEKRLAADDPEVRVLHSIGHSAAPRLFPLAWT 193
      .
177 PVLWSLLGS*.. 186
    .:|:.||
194 VLLLPLLLLQTP 205
```

[redacted]erk6.Pep check: 6430 from: 1 to: 186

[redacted]NSLATE of: mlerk6.seq check: 8999 from: 1 to: 558
generated symbols 1 to: 186.
WORKING FILE
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to: Mc6.Pep check: 7024 from: 1 to: 168

TRANSLATE of: mc6.seq check: 5844 from: 2 to: 505
generated symbols 1 to: 168.
Sequence of murine C6 (LERK-4) as derived from the genomic clone (3.5 kbp Sst1 fragment).

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgappep.Cmp
CompCheck: 1254

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	111.3	Length:	196
Ratio:	0.663	Gaps:	7
Percent Similarity:	65.190	Percent Identity:	45.570

Mlerk6.Pep x Mc6.Pep 16:31 ..

```
   1 RARANADRYAVYWNRSNPRFQVSAVGDGGGYTVEVSINDYLDIYCPHYGA  50
                        :...||       ||:::|||||||:||||:.
   1 ..................LLRGDAV.......VELGFNDYLDIFCPHYES  25

  51 PLPPAERMERYILYMVNGEG.HASCDHRQRGFKRWECNRPAAPGGPLKFS  99
     | ||.:. | : ||||: .| .|:... ..:|.||:|..| || :|::||
  26 PGPPEGP.ETFALYMVDWSGYEACTAEGANAFQRWNCSMPFAPFSPVRFS  74

 100 EKFQLFTPFSLGFEFRPGHEYYYISATPPNLVDRPCLRLKVYVRPTN.ET 148
     ||:| :|||.||||| ||..|||||...|: .:| ||||.| | ..: :.
  75 EKIQRYTPFPLGFEFLPGETYYYISVPTPESPGR.CLRLQVSVCCKESGS 123

 149 LYEAPEPI.FTSNSSCSGLGGCH.......LFLTTVPVLWSLLGS* 186
      .|.:.|:  .::|:.||: |.|       |:|  :|:|: | .
 124 SHESAHPVGSPGESGTSGWRGGHAPSPLCLLLLLLLPILRLLRVL. 168
```

Mlerk6.Seq check: 8999 from: 1 to: 797

ORKING FILE
DO NOT COPY!

to: A2.Seq check: 9214 from: 1 to: 987

HEKL
CLONE A2
SEQ REQ 1741
DIR= [JOHNSONL.HEKL]

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgapdna.Cmp
CompCheck: 6876

Gap Weight:	5.000	Average Match:	1.000
Length Weight:	0.300	Average Mismatch:	0.000
Quality:	362.8	Length:	1011
Ratio:	0.455	Gaps:	9
Percent Similarity:	56.016	Percent Identity:	56.016

Mlerk6.Seq x A2.Seq 16:33 ..

```
   1 ..........................CGGGCCCGGGCCAACGCTGAC  21
                                |  |   ||||    |   ||
 101 TGCCGCTGCTGCCGCTGCTGGCCCAAGGGCCCGGAGGGGCGCTGGGAAAC 150

  22 CGATACGCAGTCTACTGGAACCGTAGCAACCCCAGGTTTCAGGTGAGCGC  71
     ||  | || || |||||||||| |   |||||      | | |
 151 CGGCATGCGGTGTACTGGAACAGCTCCAACCAGCACCTGCGG........ 192

  72 TGTGGGTGATGGCGGCGGCTATACCGTGGAGGTGAGCATCAACGACTACC 121
               | |  ||||| |||||| |||||| | | |||||||| |
 193 ..........CGAGAGGGCTACACCGTGCAGGTGAACGTGAACGACTATC 232

 122 TGGATATCTACTGCCCACACTA.....................CGGGGCG 150
     ||||||| |||||||| |||||                     |||||||
 233 TGGATATTTACTGCCCGCACTACAACAGCTCGGGGGTGGGCCCCGGGGCG 282

 151 CCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTACATGGTGAA 200
       | |   || |  |    |    |||| |||| | ||||||||||||||
 283 GGACCGGGGCCCGGAGGCGGGGCAGAGCAGTACGTGCTGTACATGGTGAG 332

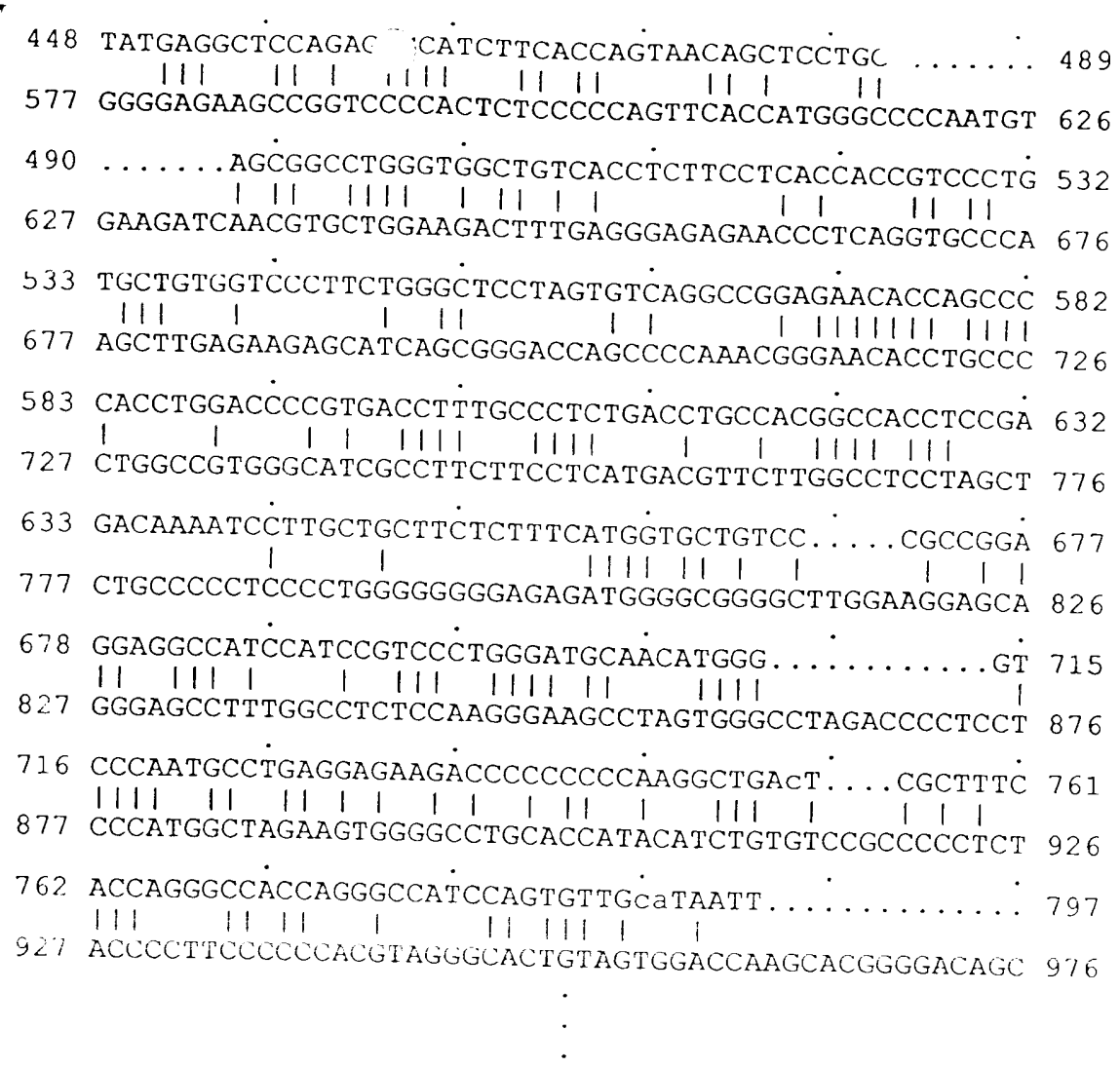
 201 TGGTGAGGGCCACGCCTCCTGTGACCACCGGCAGCGAGGCTTCAAGCGCT 250
       |  | ||| ||  | ||||  ||  | | |||   ||||||||||||||
 333 CCGCAACGGCTACCGCACCTGCAACGCCAGCCAG...GGCTTCAAGCGCT 379

 251 GGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCAAGTTCTCAGAG 300
     |||| ||||||||||||     || ||      | ||| |||||||||| |||
 380 GGGAGTGCAACCGGCCGCACGCCCCGCACAGCCCCATCAAGTTCTCGGAG 429

 301 AAGTTCCAACTCTTCACCCCCTTTTCCCTGGGCTTTGAGTTCCGGCCTGG 350
     |||||||| | || || | |||| || |||||||  |||||||    | ||
 430 AAGTTCCAGCGCTACAGCGCCTTCTCTCTGGGCTACGAGTTCCACGCCGG 479

 351 CCACGAATACTACTACATCTCTGCCACACCTCCCAACCTCGTGGACCGAC 400
     ||||||  |||||||||||||||   |   |  ||| |||||    || || |
 480 CCACGAGTACTACTACATCTCCACGCCCACTCACAACC...TGCACTGGA 526
```

0046



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          Quality:  183.3          Length:    338
            Ratio:  0.554           Gaps:      3
Percent Similarity: 61.846  Percent Identity: 61.846

Mlerk6.Seq x Mc6.Seq                    14:13  ..

     99 TACCGTGGAGGTGAGCATCAACGACTACCTGGATATCTACTGCCCACACT 148
        |||||| || || ||||||| ||||| || |||| ||||||||| |
     20 .GTGGTGGAGCTGGGCTTCAACGATTACCTAGACATCTTCTGCCCACATT 68

    149 ACGGGGCGCCGCTGCCCCCGGCTGAGCGCATGGAGCGGTACATCCTGTAC 198
        | |     ||   |||||   | ||  ||  |||    |      | |||
     69 ATGAAAGCCCAGGGCCCC...CAGAAGGCCCGGAAACCTTTGCATTATAC 115

    199 ATGGTGAATGGTGAGGGCCAC...GCCTCCTGTGACCACCGGCAGCGAGG 245
        ||||||  |  |    ||| ||   |||| |   |   |  ||      |
    116 ATGGTGGACTGGTCAGGCTACGAGGCCTGCACGGCAGAGGGGGCAAATGC 165

    246 CTTCAAGCGCTGGGAATGCAACCGGCCCGCAGCGCCCGGGGGACCCCTCA 295
        ||||  |||||||| | |||     |||    || ||     | ||  |
    166 CTTCCAGCGCTGGAATTGCTCGATGCCTTTTGCCCCTTTCAGCCCTGTTC 215

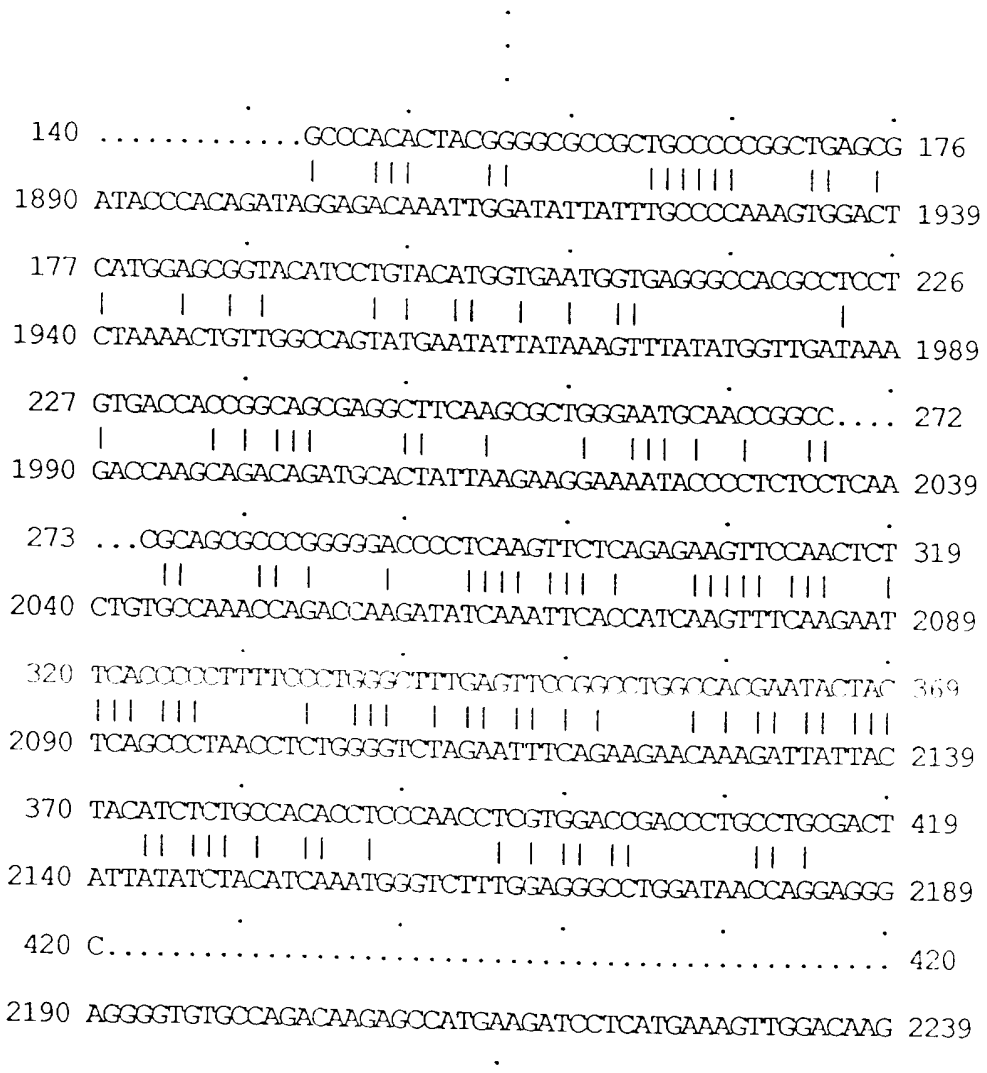
    296 AGTTCTCAGAGAAGTTCCAACTCTTCACCCCCTTTTCCCTGGGCTTTGAG 345
          |||||||| ||| | || | || ||| |||||  | ||||||||||||
    216 GATTCTCAGAAAAGATTCAGCGCTACACACCCTTCCCGCTGGGCTTTGAG 265

    346 TTCCGGCCTGGCCACGAATACTACTACATCTCTGCCACACCTCCCAACCT 395
        |||  ||||||  |    |||||||||||||| |   | ||||  |
    266 TTCTTGCCTGGAGAGACTTACTACTACATCTCGGTGCCGACTCCGGAGAG 315

    396 CGTGGACCGACCCTGCCTGCGACTCAAGGTTTATG... 430
        | |||    |||||| ||||| |||| | ||
    316 TCCTGgCCG...GTGCCTGAGACTCCAGGTGTCTGTCT 350
$
```

```
          Quality:  104.9          Length:    411
            Ratio:  0.373           Gaps:      1
Percent Similarity: 39.858  Percent Identity: 39.858

Mlerk6.Seq x Lerk5.Seq                14:01  ..
```



HEK/ELK Meeting Minutes

B. Davison in the absence of Doug Cerretti.

Nicole Nelson summarized the recent screening of a murine embryonic cDNA library with a combination of LERKs(A2,C6) plus GSK beta kinase(Fred Fletcher's probe): Hybridization conditions were 42 C in 50% Stark's followed by washes at 63 C, 0.1X SSC.

13 initial positives were obtained of which 4 did not repeat; Nicole will rescreen these using a less stringent wash protocol. Currently, the sequence analysis indicates that the collection contains at least1 gsk Beta clone, 1 gsk Beta-like clone and interestingly, a new LERK, LERK-6.

All four cysteine residues are conserved while inspection of the carboxy terminal portion of the sequence indicates that LERK-6 fits into the GPI-linked class(similar to LERKS 1,3 and 4). The amino terminus of the protein is apparently lacking about 20 or 25 amino acid residues in the current clone. In the binding region, the LERK-6 DNA sequence displays about 70% identity with A2(LERK 3) corresponding to 288 bp of overlap.

Efforts are now being directed at identification of a source to obtain the human homologue.

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American Type Culture Collection
12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Immunex Corporation
Attention: Stephen L. Malaska
Legal Affairs Department
51 University Street
Seattle, WA 98101

Deposited on Behalf of: Immunex Corporation (Docket No. 2826)

Identification Reference by Depositor:	ATCC Designation
Recombinant phage lambda gt10 vector, clone lambda 13M LERK-6 (murine)	75829

The deposit was accompanied by: __ a scientific description . a proposed taxonomic description indicated above.

The deposit was received by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Bobbie A. Brandon

Date:

Bobbie A. Brandon, Head, ATCC Patent Depository

Form BP4/9